CHAPTER 10

Summary, general discussion and future perspectives

SUMMARY

Patients with primary and secondary immunodeficiencies are at increased risk of contracting common and/or opportunistic infections [1, 2]. Moreover, these infections more often follow a complicated course, with increased morbidity and mortality [1, 2]. One of the interventions to prevent or mitigate the course of infections in these patients is vaccination. However, paradoxically, for an optimal response following vaccination an adequately functioning immune systems is required (also see introduction to this thesis).

In order to make recommendations on the usefulness of influenza vaccination in patients with primary and secondary immunodeficiencies, we investigated the humoral and cell-mediated immune (CMI) responses following influenza vaccination in these patients. Moreover, the timing of vaccination in relation to immunosuppressive treatment was studied as well as a strategy to optimize the immune response to influenza vaccination: administration of a second, booster, influenza vaccination.

Following chapter 1, where we summarized the history of the discovery of vaccination, the immune response to influenza vaccination, and the primary and secondary immunodeficiencies that are addressed in the thesis, part 1 describes the vaccination studies that we performed in patients with humoral primary immunodeficiencies (hPID). Chapter 2 addresses the humoral immune response following inactivated trivalent subunit influenza vaccination in patients with hPID. Following vaccination, as expected, hPID-patients developed humoral responses that were clearly inferior to those found in healthy controls (HC), as measured by geometric mean titers (GMTs), fold-increase in GMT, and seroprotection rates. In contrast to HC, who responded to all three influenza strains, patients were able to respond with a significant rise in GMT only for the A/H1N1-strain. Moreover, previous vaccination and treatment with intravenous immunoglobulin (IVIg) did not result in higher postvaccination GMTs or higher rate of seroprotection (defined as titer ≥40).

Since CMI responses are also of importance for the clearance of influenza from infected individuals, in the study presented in chapter 3 we investigated the cell-mediated recall responses following influenza vaccination in patients with common variable immunodeficiency (CVID), a subgroup of the hPID-patients that were included in the study described in chapter 2. Although cellular immune responses are affected in patients with CVID at many different levels, the cellular response following influenza vaccination had never been addressed before by others. We found impaired recall responses following influenza vaccination in the CVID-patients when using interferon (IFN)γ-ELISpot. Flow cytometry with intracellular cy-

tokine staining revealed a decrease in influenza-specific IFNγ and tumor necrosis factor (TNF)α producing $CD4^+$ and $CD8^+$ T-cells in patients following vaccination compared to baseline, while HC demonstrated an increase in influenza-specific cytokine producing T-cells. In vitro activation-induced cell death of $CD4^+$ and $CD8^+$ T-cells might be the explanation for this fall in T-cell numbers after influenza vaccination as increased pre-activation of T-cells in vivo has been demonstrated in CVID patients.

Part 2, addressing immune responses to influenza vaccination in patients with secondary immunodeficiencies, in particular those suffering from auto-inflammatory rheumatic diseases (AIIRD), starts with two studies on influenza vaccination in patients with systemic lupus erythematosus (SLE). The first focused on the humoral and CMI responses before and after influenza vaccination (chapter 4). SLE patients showed a decreased antibody response to A/H1N1 and A/H3N2, compared to HC. Cell-mediated influenza-specific responses were also found to be lower in SLE-patients in comparison with HC. Using FACS analysis, SLE-patients showed increases in frequencies of influenza-specific $CD4^+$ T-cells for fewer cytokines following influenza vaccination as compared to HC. Impaired cell-mediated influenza-specific responses were associated with the use of prednisone and/or azathioprine. The second study (chapter 5) investigated a strategy to improve the outcome of vaccination in SLE-patients, since an earlier study (chapter 4) demonstrated sub-optimal humoral responses in these patients. In this re-vaccination study, the efficacy of administration of a second, booster, influenza vaccination four weeks after the first one was studied. In contrast to previous data, with in part other influenza strains, this study did not find differences in the influenza-specific antibody responses between SLE patients and HC after the first vaccination. The booster vaccination did not result in a further rise in GMT, seroconversion rates or seroprotection rates, except for SLE patients who were not vaccinated in the previous year, who tended to have an additional increase in GMT and seroconversion rate for the A/H1N1 influenza-strain.

Chapter 6 describes a study that was initiated to investigate the influence of treatment with rituximab, a B-cell depleting anti-CD20 monoclonal antibody, on the antibody response following influenza vaccination in patients with rheumatoid arthritis (RA). In B-cell depleted individuals no influenza-specific antibodies were produced following influenza vaccination. This was expected, as influenza vaccine antigens are considered (in part) to be neo-antigens. The antigens are yearly adjusted to the changes in the influenza virus caused by antigenic drift. Vaccination within 4 to 8 weeks after administration of rituximab showed no humoral response at all. However, 6 to 10 months following rituximab therapy, when recurrence of B-cells in the peripheral blood occurred, a significant, but still hampered,

antibody response to both A-strains in the vaccine could be demonstrated.

Within the same study population that was studied in chapter 6, the CMI responses before and after influenza vaccination (using IFNγ-ELISpot, proliferation assays and flowcytometry with intracellular cytokine staining) were determined, as described in chapter 7. Before vaccination, flow cytometry demonstrated that polyclonal and influenza-specific CMI responses were reduced in RA-patients treated with rituximab. Moreover, influenza-specific CMI responses following influenza vaccination were hampered.

Finally, part 3 presents the systematic literature review (SLR) (chapter 8) that was performed to form the basis of evidence-based recommendations on vaccination in patients with auto-immune inflammatory rheumatic diseases (AIIRD).

After the multidisciplinary expert committee commissioned by EULAR defined the AIIRD, vaccines and immunomodulating drugs to be included in the search, eight key questions were answered. The EULAR recommendations for vaccination in adult patients with AIIRD based on this SLR and expert opinion, using Delphi voting, can be found in chapter 9. Although more research is needed, in particular regarding incidence of vaccine preventable infections (VPI), harms of vaccination and the influence of (new and established) immunomodulating agents on vaccination efficacy, 13 recommendations were formulated. Moreover, a research agenda was proposed.

GENERAL DISCUSSION

In the studies described in this thesis, we evaluated humoral and CMI responses following influenza vaccination in patients with primary and secondary immunodeficiencies in order to determine the usefulness of influenza vaccination in these categories of patients. Moreover, (influenza) vaccination can be used as a tool to measure the residual immune response as the resultant of the immunosuppression in immunocompromised patients. When administrating identical antigenic stimuli such as influenza vaccine, the immune response is mainly dependent on host factors.

Occurrence of influenza in primary and secondary immunodeficiencies

Influenza infection is clinically asymptomatic in 30-50% of infected cases. It generally presents with an acute illness characterized by fever, chills, sore throat, myalgias, headache and fatigue. Symptoms last an average of 7 days [1, 2]. However, gastro-intestinal symptoms may be most prominent in children, but during the novel influenza A/H1N1 pandemic in 2009 with the influenza virus strain A/H1N1/California/2009 also in adults abdominal complaints frequently occurred as one

of the presenting symptoms (3).

Influenza is usually a self-limiting disease, but complications may occur in particular in young children, elderly and immunocompromised hosts. Common complications are exacerbations of underlying respiratory or heart disease. Viral pneumonia may occur as well as secondary bacterial pneumonia [1-3]. The last pandemic confirmed the vulnerability of young children, patients with underlying cardiopulmonary diseases and immunocompromised patients [3, 4].

For the specific patient groups addressed in this thesis, however, little is known on the occurrence, morbidity and mortalitiy of influenza infection. Patients with hypogammaglobulinemia (chapter 2 and 3) frequently experience bacterial upper and lower respiratory tract infections [5-7], and it seems logical to assume that the risk of contracting influenza and its morbidity and mortality are increased in patients with hypogammaglobulinemia, because humoral immunity following influenza vaccination correlates with protection from or mitigation of influenza infection [8]. Moreover, an increasing amount of data supports the role CMI responses in the clearance of influenza [9, 10]. CMI responses are hampered in patients with CVID [5, 11-20]. However, no data are available regarding viral pathogens causing respiratory infection is these patients. Therefore, not only the mortality and morbidity resulting from influenza infection remains unknown, it is also impossible to estimate the efficacy of influenza vaccination on clinical endpoints in these patients.

In patients with secondary immunodeficiencies (SLE and RA, treated with immunosuppressive medication) that we studied in chapter 4, 5, 6, and 7 it has been shown that morbidity and mortality from pulmonary infections is increased, but no studies assessed the causative micro-organisms that led to these pulmonary infections. Two studies that included elderly patients with an increased risk of contracting influenza, among whom patients with rheumatic diseases and vasculitis, found an increased risk for hospital admission for either pneumonia or influenza and for death, compared to low-risk elderly [21, 22]. A subgroup analysis of patients with rheumatic diseases or vasculitis, however, was not performed. Still, patients with rheumatic diseases should be considered at increased risk of influenza and a complicated course of influenza.

Efficacy of influenza vaccination

The aim of vaccination is to reduce the morbidity and mortality, directly or indirectly provoked by the vaccine-preventable disease. Therefore, studies evaluating the efficacy of vaccination should ideally use clinical endpoints. In case of influenza vaccination, the reduction of microbiologically confirmed influenza

infection, the occurrence of influenza-like illness (ILI), influenza-related hospital admission, pneumonia and death have been used as clinical endpoints of studies evaluating the efficacy of influenza vaccination.

On the other hand, ILI can also be caused by many infections other than influenza and for a long time it has been hard to confirm influenza microbiologically, because no sensitive tests for influenza were available (only viral culture and serology). Moreover, vaccine efficacy also depends on the homology between the influenza-strains in the vaccine and the actually circulating influenza-strains. Since influenza strains yearly undergo an antigenic change, efficacy of influenza vaccination varies per year and per strain [1, 2]. Therefore, studies investigating efficacy of influenza vaccination on clinical endpoints should investigate their study participants for influenza on a regular basis with a sensitive microbiological test, include large numbers of participants and cover multiple influenza seasons. Such a study is difficult to accomplish, and therefore many studies assessed the efficacy of influenza vaccination by determining surrogate outcome measures.

The most important surrogate parameter is the humoral immune response as determined by hemagglutination inhibition assay (HIA), because this parameter has been shown to correlate with protection from influenza. Hemagglutination inhibition (HI)-titer ≥40 is protective in young healthy adults, and in 90% of healthy young adults HI-titers rise to ≥40 after vaccination [8]. In elderly and most immunocompromised persons the humoral response following influenza vaccination is suboptimal [23-26]. Moreover, it has never been demonstrated which HI-titer should be strived for in elderly or immunocompromised patients. For example, elderly with very high HI-titers might not be protected from influenza [27-29].

The fact that despite so-called protective antibody levels individuals might not be protected from influenza might be due to an impaired cellular response. The influenza-specific cell mediated immune (CMI) responses following influenza vaccination are important for the clearance of influenza after infection [10, 27, 29] and can be induced by influenza vaccination, in particular when using whole inactivated virus (WIV)-vaccine or live attenuated influenza-vaccine. In children the production of IFNγ-production has been shown to correlate with protection from influenza [30]. In elderly granzyme-B (as a measure of the effector mechanism of cytotoxic T-lymphocytes), and the ratio between IFNγ and interleukin(IL)-4 (as a measure of the balance between the Th1- and Th2-response) was demonstrated to correlate better with protection from clinical influenza than the HI-titer [27, 29].

In the studies reported in this thesis we evaluated both humoral and CMI responses to determine the efficacy of influenza vaccination in our patient groups with hypogammaglobulinemia, SLE and RA. The humoral immune response was deter-

mined with the generally accepted HIA, the CMI response by using IFNγ-ELISpot, flow cytometry with intracellular cytokine staining for IFNγ, IL-2 and tumor necrosis factor (TNF)α and CFSE-dye dilution proliferation assay. IFNγ-ELISpot has been demonstrated to be sensitive, but only determines the production of one cytokine by PBMC, a mixture of different white blood cells. The advantage of flow cytometry with intracellular cytokine staining is its capability to determine the production of several cytokines in well defined subsets of PBMC, but it is a labour-intensive technique and less sensitive than ELISpot [31]. Finally, the CFSE-dye dilution proliferation assay is the most functional assay, assessing an important aspect of the CMI response: the capability of predefined cell types to proliferate in a clonal fashion in response to a specific antigen. However, none of these parameters of cellular immunity have been shown to correlate with protection from influenza in the investigated study populations.

In patients with hypogammaglobulinemia, especially in patients with CVID, hampered humoral and CMI responses were recorded (chapter 2 and 3). Influenza vaccination did not lead to an increase in seroprotection rates. Pre-existent anti-influenza HI-titers might have resulted from earlier infections or treatment with IVIg. SLE patients seem to have humoral responses following influenza vaccination comparable to HC, but might benefit from a second, booster vaccination if not previously vaccinated. CMI responses following influenza vaccination in SLE-patients did increase, but were lower compared to HC, before as well as following vaccination (chapter 4 and 5). Finally, RA patients treated with rituximab, will not respond to influenza vaccination with a rise in HI-titer or increase in seroprotection rates, unless the interval after the last administration of rituximab is at least 6-10 months. Also these RA patients demonstrated reduced CMI responses at baseline and following influenza vaccination, indicating an important role for B-cells also in CMI responses.

Clinical implications of the findings in this thesis

It is hard to determine the clinical implications of influenza vaccination in the patients categories assessed in this thesis realising that the burden of influenza in these patients is unknown. However, as it is generally accepted that immunocompromised patients "not-otherwise-defined" are at increased risk of contracting influenza and of a complicated course of influenza, our studies do have implications.

The reduced humoral as well as to CMI responses to influenza vaccination in hypogammaglobulinemia patients, especially CVID patients, requests for additional measures to prevent influenza in these patients. Influenza vaccination should be

offered to persons caring for or residing with patients with hypogammaglobulinemia and post-exposure prophylactic antiviral treatment should be considered in these patients. When influenza is suspected, these patients should be treated empirically.

Recommendations for vaccination in patients with AIIRD (chapter 9), based on a systematic literature research (chapter 8), have recently been developed for the European League against Rheumatism (EULAR). The articles regarding influenza vaccination in patients with SLE, and RA patients treated with rituximab, performed by our research group (chapter 5 and 7), contributed to these recommendations. Because of the increased risk of pulmonary infections in AIIRD patients, they should be offered influenza vaccination. A booster can be considered in previously unvaccinated patients, as also supported by the reduced response in AIIRD patients vaccinated with the novel non-adjuvanted A/H1N1-vaccine [32]. Furthermore, vaccination of patients treated with rituximab should be administered before the start of rituximab, since rituximab severely hampers the immune response to influenza vaccine.

Besides recommendations on influenza vaccination in patients with AIIRD, also all other in Europe available vaccines were addressed in the EULAR recommendations on vaccination in patients with AIIRD (chapter 9). The next, last, step in the assessment of the clinical implications of vaccination is of course the implementation of these recommendations. Several studies show that, despite recommendations and guidelines supporting influenza vaccination in patients with AIIRD, only a small proportion of these patients do receive influenza vaccination [33-36]. Therefore, care givers should be informed about the recent EULAR recommendations for vaccination in patients with AIIRD during symposia and conferences. For practical implementation general practitioners, and doctors and nurses specialised in the care for patients with AIIRD, need to offer and actually administer the vaccines. One Dutch study revealed that, despite an indication for influenza vaccination according to the guidelines, 262 of 595 patients were not vaccinated. Half of them was not advised about the vaccination, or was even advised to restrain from vaccination. In contrast, 86% of patients with an indication for influenza vaccination according to the guidelines was vaccinated when advised to [37].

FUTURE PERSPECTIVES

Although some questions have been answered in the studies in this thesis, and practical recommendations based in the currently available evidence have been formulated regarding vaccination in patients with AIIRD, still many questions remain unanswered and some additional questions came up. Therefore, there are many opportunities for further research.

Epidemiology of influenza and its complications in patients with primary immunodeficiencies and AIIRD

As discussed in the general discussion, it is unknown what the extent is of influenza-related morbidity and mortality in patients with primary immunodeficiencies and AIIRD. Without these data, the efficacy of and also the indication for influenza vaccination remain largely unknown in these patient groups. Also cost-efficacy can not be addressed without this knowledge. Studies investigating the frequency of influenza infection and the morbidity and mortality associated herewith should be performed. Serological studies before and following the influenza season 2009-2010, when the novel influenza A/H1N1 occurred, might provide insight in the epidemiology, although the occurrence of a pandemic is exceptional and because of the large proportion of patients vulnerable for a new influenza virus strain reduces the possibility to extrapolate these data to seasonal flu. Another option would be to determine reference centres treating patients with primary immunodeficiencies and AIIRD that investigate the incidence of particular vaccine-preventable infections, among which influenza, during a predefined period. Those reference centers can also evaluate the safety of vaccinations in patients with AIIRD, as limited (however reassuring) data are available, in particular about safety of vaccination in patients treated with newer biologic therapies and/or active disease.

Correlates of protection for influenza

Since large studies assessing clinical endpoint will remain hard to perform in subgroups of immunocompromised patients, there is a need for good correlates of protection from influenza. Not only should those parameters be determined in healthy adults, also correlates of protection should be defined for immunocompromised patients, since currently it is uncertain if a HI-titer ≥40 also correlates with protection in these patients. It should also be investigated which parameters for CMI responses following influenza are protective. A good candidate could be granzyme-B [27, 29, 38]. Standardised assays for granzyme-B have recently become available. Also the IFNγ/IL-4 ratio should be further explored [27, 29, 38].

More effective immunization of patients with primary immunodeficiency or AIIRD

Since influenza-specific immune responses are hampered in the patient groups we investigated in this thesis, more effective immunizations are needed to provide protection from influenza. For example, adjuvants can be added. Alum is well known for its role as an adjuvant. Since it shifts the immune response towards a Th2-response, it leads to better antibody production [39]. Also oil-in-water emul-

sion adjuvants, i.e. MF59 and AS03, which enhance the recruitment and activation of antigen presenting cells and therefore induces stronger immune responses following influenza vaccination [40], might be used. Moreover, addition of MF59 leads to cross-protection against influenza-strains that were not included in the vaccine [41]. MF59-adjuvanted influenza A/H1N1v vaccination did not result in an increase in disease activity scores in patients with RA, SLE, or ankylosing spondylitis [42]. A stronger Th1 response can be induced by using whole virus vaccines, unfortunately these are also more reactogenic [38, 43]. Furthermore, antigens can be offered within a constructed virus envelope, so called virosomes or virus-like particles, leading to both humoral and CTL-responses [43-48]. Besides hemagglutinin (HA) and neuraminidase (NA), internal proteins of influenza virus, that are more conserved than HA and NA, can the offered in the virosomes for a better CMI response [48]. Stimulation of Toll-like receptors along with the administration of influenza antigens might lead to better Th1 responses and potentially better protection from influenza [49;50]. Finally, intradermal administration of influenza vaccine could be an option to ameliorate the immune response following influenza vaccination, since resident dendritic cells in the dermis are able to stimulate innate immunity thereby increasing the adaptive immune response to vaccination. This has been shown to be safe and feasible in immunocompromised patients in one study, but CMI responses were not assessed [51].

For all these strategies it remains important to realise that the immune system is stimulated more profoundly and therefore the chance of side effects is increased. In particular for patients with (an increased risk of) auto-immune diseases, the benefit of better influenza-specific immune responses may not outweigh the risks of developing auto-immune phenomena in patients with primary immunodeficiencies or of disease flares in patients with pre-existent AIIRD.

Implementation of the recently developed recommendation for vaccination of patients with AIIRD

Finally, the evidence-based recommendations that we developed need to be implemented, so that all patients with AIIRD can take advantage of the available vaccines. Little has been published on implementation of influenza vaccination in these specific patient groups. The available evidence shows increasing coverage when a positive vaccination advise is given by the treating physicians and nurses [37].

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